LAB-BASED FILTRATION SYSTEM IMPROVING RECOVERY AND REDUCING TIME TO DETECTION FOR DIAGNOSIS OF SEPSIS

by

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Abstract

Rapid and accurate detection of a blood stream infection is critical for the proper management of patients displaying symptoms of sepsis. Current blood culture systems are both time consuming and may yield low recovery in the presence of antibiotics, delaying needed results for effective sepsis management. To improve recovery rates and time to detection, a novel sample preparation and culture device system was created. The design concentrates the microorganisms into a small volume, while removing antibiotics and inhibitory blood components. This system allows for both increased recovery and decreased time to detection, granting rapid and accurate detection of blood stream infections to help clinicians diagnose septicemia.

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Prof. Hai-Quan Mao, Ph.D., Johns Hopkins University

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Table of Contents

Table of Figures

Introduction

Sepsis is considered among the most significant healthcare issues due to high incidence and high mortality rate.[1] The mortality rate is especially concerning, as sepsis contributes to 33-55 percent of all hospital deaths, while occurring in 10 percent of all hospital patients.[2] Furthering its case as a leading public health concern, sepsis accounted for over $20 billion (5.2%) of US hospital costs in 2011.[3]

Although sepsis can affect anyone, certain groups are at an increased risk. Those with other health problems, such as diabetes, and those who have recently been hospitalized or had an invasive medical procedure are particularly vulnerable. Also at greater risk are those with compromised immune systems, such as patients with HIV or cancer, very young babies, and the elderly.[4] These risk factors stem from the limited ability to fight infection, increasing the chances that an infection progresses to sepsis in these patient populations.

Sepsis, a clinical syndrome characterized by a dysregulated host response to infection, often leads to organ failure and ultimately death.[5] This dysregulated response may arise due to the presence of an infectious microorganism in numerous bodily tissues, although blood stream infections are among the most common causes. Sepsis is considered severe when signs of organ dysfunction or altered cerebral function appear. If blood pressure drops to dangerous levels, the diagnosis of septic shock is then made.[1] The infection causing sepsis is usually associated with illness, but can also arise from an injury or follow a routine surgery.

If a doctor suspects sepsis, a number of tests will be run to diagnose the condition. These tests look for a number of markers including, a high or low white blood cell count, a low platelet count, acidosis, altered organ function, and infectious microorganisms within

bodily fluids.[6] While cell counts and altered organ function can help lead to a sepsis diagnosis and general treatment to keep vital organs functioning, isolating bacteria from the infection source is necessary to determine antibiotic susceptibility. Determining to which antibiotics a particular infection is most susceptible allows clinicians to employ the most effective treatment to fight the infectious microorganism.[7]

The current process for determining antibiotic susceptibility begins by taking a blood sample from the patient for blood culture. Blood culture involves the collection, incubation, growth, and detection of viable microorganisms within a blood sample. Following the culture, the microorganism must be isolated and then have identification and antibiotic susceptibility testing performed. This process can be exceedingly time-consuming, thus delaying the time before optimal treatment. Figure 1, below, shows a sample timeline from collection to the needed antibiotic susceptibility result.

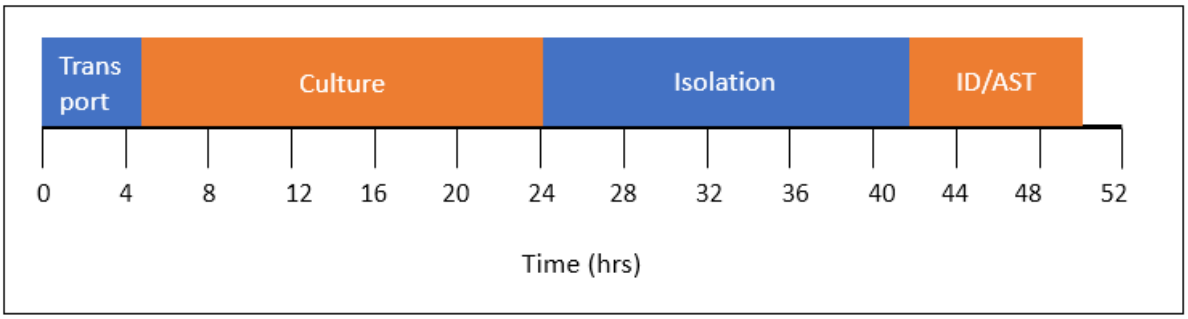

Figure 1. Timeline of traditional blood culture from collection/transport to identification (ID) and antibiotic susceptibility testing (AST).

The most widely used systems for blood culture are BACTEC (Becton Dickinson) and BacT/Alert (bioMerieux). Each system uses its own proprietary media, detection system, and algorithms. However, both follow a similar protocol: A roughly 10-mL blood sample is taken from a patient and inoculated into a bottle containing growth media; the sample is transported to a lab and placed into a machine that incubates the sample and employs

continuous monitoring to alert when microbial growth has been detected. After a positive result, the bacteria/fungi must be isolated to allow for downstream processing steps leading to identification and antimicrobial susceptibility testing. Although the specifics of identification and antimicrobial susceptibility testing are beyond the scope of this work, this isolation step is extremely relevant since it arises due to the nature of current systems and can add upwards of 24 hours before antimicrobial susceptibility testing can be performed.

Current automated blood culture systems, such as the BACTEC and BacT/Alert, have an extremely simple workflow and minimal risk for contamination. This is due to the closed nature of these systems. Since a sample is collected and inoculated directly into a sealed bottle, nearly all contaminants originate from the patient's skin. This allows minimization of contamination via sterilization of the draw site, and also allows clinicians to generally recognize coagulase-negative staphylococci as potential contaminants.[8] In addition; these systems provide high recovery in the absence of antibiotics. In the simplest sense, high recovery means that the bacteria present in a patient's blood are captured in a viable state and grow until detection. These systems yield high recovery because there is almost no area for a loss of viable organisms; the blood is transferred directly to a culture bottle, and then viable organisms should be sustained within the growth media. However, when antibiotics are present recovery is often decreased, since the inactivation of antibiotic by charcoal or resin is time dependent, many previously viable organisms may be lost before the antibiotics are effectively neutralized.

These systems for growing and detecting microorganisms have been used for decades and while they have many positives, there are certainly drawbacks to such systems. Chief among them is the amount of time it takes to obtain a result. The time to determine the

presence of a microorganism can take 12 – 72 hours depending on the organism and whether or not the patient is on an antibiotic regimen. Further, antibiotics can significantly reduce recovery, even in systems using activated charcoal or resins to neutralize antibiotics.

Some aspects of these problems are inherent of the nature of current blood culture systems. A relatively large volume of blood (~10 mL) is needed to ensure viable organisms are captured, and then once growth media is added, the increased total volume significantly reduces the initial bacterial concentration. Since the starting concentration is dilute, it takes more time for a sample to reach a sufficiently high concentration and to be detected. A simple blood culture bottle may contain resins to effectively neutralize some antibiotics; however, these systems do not effectively eliminate all antibiotics from the microorganism environment, which inevitably leads to a decrease in susceptible cell viability. Further, these systems culture the pathogenic organism within a mixture of growth media and the patient's whole blood. The presence of the various blood components can inhibit the growth rate of some organisms, but more importantly, downstream processing, such as antimicrobial susceptibility testing, require pure organisms free from blood components. Currently, to acquire pure organisms from a positive blood culture bottle, a subculture is taken from the bottle, inoculated on plated media and allowed to grow. This extra step can add more than 24 hours between a positive test result and knowing how to effectively treat the infection.

The mechanism of detection of aerobic microorganisms in the BACTEC automated blood culture system is based on CO_2 sensing. Microorganisms undergoing aerobic respiration use oxygen to produce ATP. Proteins and sugars are often consumed as reactants, while CO_2 is generated as a product of the reaction. Although this is a simplified view of respiration, it is the basis for the CO_2 detection mechanism within BACTEC systems. As a

sensor within the system indirectly detects the amount of CO_2 present at a given time, an algorithm analyzes the data, combines this data with previous time-points, and determines whether there is growth occurring within the culture bottle. This process is similar for other automated culture systems, such as BacT/Alert, although the method in which CO_2 is indirectly measured differs between the two.

Project Aims

One specific aim of this project is to create a bacterial enrichment system, which serves to increase the recovery of viable microorganisms, from the point of sample collection through growth and detection. Current blood culture methods yield high recovery in the absence of antibiotics; however, many patients have antibiotics administered before a blood culture specimen is taken. The proposed system must mitigate the effect that antibiotics present in the patient's bloodstream may have on the viability of susceptible organisms within the culture device.

Another specific aim is for this system to enable a decrease in total time before an antimicrobial susceptibility result is obtained. Current blood culture systems can take from 12-72 hours to determine the presence of a pathogen. After the presence of a pathogen is confirmed, it can take up to another 24 hours to prepare the specimen for identification and antimicrobial susceptibility testing. Since, over the first six hours following hypotension onset, there is a ~7% increase in mortality for every hour before effective antimicrobial treatment begins, decreasing the time to result by hours will significantly decrease mortality.[9]

Approach

Overview

To achieve the two specific aims of this project, it is necessary to design a system from the point of sample collection through sample preparation for identification and antimicrobial susceptibility testing. This is achieved by modifying the steps of traditional blood culture, shown above in Figure 1. Figure 2, below, showcases the modified steps.

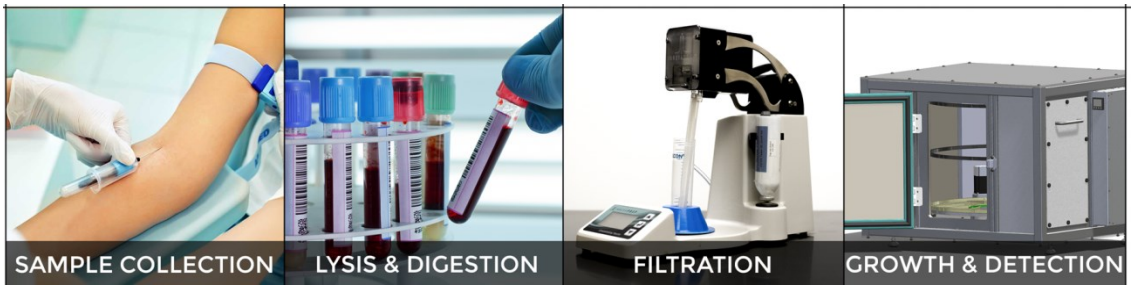

Figure 2. Modified blood culture steps for increasing recovery and decreasing time-to-detection (TTD).

These modified steps allow for a number of improvements over the traditional blood culture method. The introduction of a selective lysis step will improve recovery by freeing any viable microorganisms that are engulfed by white blood cells. Meanwhile, the filtration step will enable the complete removal of antibiotics AND inhibitory blood components, while also concentrating the microorganisms into a small volume. Further, filtering and concentrating the samples allows for reconstitution in a small volume of pure media, simplifying downstream processing. Since only ~10% of infections are mixed[10,11] *i.e.* contain more than one pathogenic microorganism, in roughly 90% of cases filtration will allow the bacteria to grow in a pure culture, enabling the complete removal of an up to 24-hour subculture step prior to the start of identification and antimicrobial susceptibility testing.

Recovery

In order to increase viable organism recovery, antibiotics must be neutralized, whether through chemical or physical means. This project approaches this challenge by using direct physical separation, via filtration, to remove antibiotics from the environment of the microorganism of interest. By using a filter with a pore size small enough to retain all potentially infectious microorganisms, and large enough to allow antimicrobials within the sample to pass, it is possible to completely eliminate antibiotics from the environment around the infectious microorganism. Further, this method also eliminates blood components, such as complement, which may inhibit microbial growth.

While this method removes inhibitory factors and antibiotics from the sample, it also removes blood factors, such as Component A and Component B, which are essential for some organisms.[12] This will reduce recovery of a small subset of organisms and thus the grow media must be supplemented with the necessary factors.

Time to Detection (TTD)

In order to decrease the time to detect the presence of an infectious microorganism, a few approaches may be taken. One method is to concentrate the microorganisms in a reduced volume. Microorganisms can be concentrated in a reduced volume by filtering the initial sample, and then adding a small volume of growth media to the microorganisms trapped on the filter. Traditional blood culture has a total volume of around 40 mL, whereas filtering and then adding growth media allows for volumes <1 mL. This 40-fold volume decrease equates to ~5.3 doublings (*i.e.* $2^{5.3} \approx 40$), meaning that this volume reduction alone can reduce the time to detection by 5.3 generations. This saves about 2 hours for fast growing organisms

such as *E. coli* (~23 min/gen), however, the effect is much more significant for slow growing organisms such as *C. albicans* (~73 min/gen), saving roughly 6.5 hours.

Another method to decrease TTD is by employing a more sensitive detection mechanism. An indirect CO_2 sensor is the standard detection mechanism; however, by removing blood components from the microbial environment, it becomes possible to explore other chemical and physical detection methods. Two detection mechanisms; one chemical: resazurin, and one physical: turbidity; are chosen to be compared to traditional CO_2 sensing.

Resazurin is a weakly fluorescent blue dye, which is reduced to the red, highly fluorescent, resorufin. As cells proliferate in the presence of resazurin, it is chemically reduced and can be detected using a fluorescence detector. Resazurin was chosen since the cells must remain fully functional for downstream testing and other assays, such as MTT, can impact the electron transport chain leading to cell death.

Turbidity is the cloudiness of a fluid caused by a large number of individual particles in suspension. A nephelometer, which employs a source beam and a detector 90° of the source beam, grants a relative measure of the turbidity of a solution. The signal received by the detector is a function of the particle density, as well as the shape, color, and reflectivity of the particles. As the cells propagate the particle density of the growth media solution increases, allowing one to take successive readings to develop a growth curve and determine positivity.

System View

The above approaches for improving recovery and TTD both rely on one important factor: isolating microorganisms from a sample of whole blood. Common methods to attempt

this include centrifugation, filtration, and surface capture/antibody techniques. Generally, surface capture techniques do not work with a broad spectrum of organisms, while centrifugation-based methods rely on more complicated processing steps. Therefore, a filtration-based method is pursued, since it allows for a simple disposable device, needing only a source of vacuum to supply the force for separation.

Filterability of a 10-mL sample of whole blood depends on a number of factors. Some of these factors can be designed and controlled, such as the lysis and digestion methods, and filter membrane properties. Other factors, such as patient blood composition (white cell count, medications, clotting factors, etc.) cannot be controlled. Further, microorganisms must remain viable during transport to the lab for filtration.

A pre-developed media is used as the basis of a transport media, while filter membrane materials and coatings must be investigated to maximize filterability. Since transport time to the lab can vary greatly among hospitals, it will be important to test how long microorganisms remain viable and the digested blood remains filterable.

System Development and Testing

Overview

The approach to developing this system can be broken down into a few key components. The first component is a media capable of lysing blood cells, while sustaining the viability of the microorganisms of interest. This media must also maintain microorganism viability in the presence of antibiotics during the period of transport between collection from the patient and filtration in the lab. Once in the lab, the media must allow for filtration of the entire volume of collected sample.

The next component for this system is filtration geometry. Filtration was initially carried out using a commercially available preassembled vertical filter with a proprietary peristaltic pump. However, a preassembled filter with proprietary pump connections offers little flexibility, so the geometry and adaptability of different filter holders was evaluated. While the geometry and customizability of the filter holder was considered, the critical aspect of the filtration setup is the filter membrane. Filterability while using a number of commercially available membrane materials was evaluated.

Once the sample has been filtered, it is necessary for the organisms to grow in order to be detected, so it is necessary to develop a nutrient rich growth media that supports all of the clinically significant organisms. A commercially available media is the basis of the growth media formulation, although additions are necessary to ensure certain fastidious organisms are capable of growth. As the organisms within a sample grow, it is important to have a sensitive detection system in order to minimize TTD. The current standard for detection is indirect CO_2 detection, which means the developed system should show

sensitivity greater than this standard. The sensitivities of two other detection systems are compared to indirect CO_2 detection.

Transport Media

The first piece of this approach is a transport media capable of allowing selective lysis of the blood cells, while preserving viable microorganisms. This media is based on a commercially available growth media; however, the purpose of this media is to simply allow transport, so the nutrients were reduced to the minimum required for viability. This reduction in nutrients is necessary since the mechanism of certain antibiotics, such as Antibiotic E, inhibits the cell wall synthesis of replicating bacteria. Supplying minimal nutrients to the organism will cause it to replicate more slowly, thus making it less susceptible to this type of antibiotic.

Component C was added to the transport media not only to serve as an anticoagulant, but also to inactivate Class A antibiotics. Table 1, below, shows the viability of *Pseudomonas aeruginosa* when it is exposed to peak serum levels of Antibiotic A, a common Class A antibiotic, within the transport media containing varying concentrations of Component C.

Table 1. *P. aeruginosa* viability in varying Component C concentrations

Component C concentration level	Presence of Antibiotic A	Fraction viable cells remaining after 4 hours of incubation
1	No Antibiotic A	**1.3**
		1.3
		1.1
1	Peak Serum Antibiotic A	**0.4**
		0.3
		0.6
2	Peak Serum Antibiotic A	**0.7**
		0.4

		0.5
3	Peak Serum Antibiotic A	**1.1**
		1.2
		1.2

The above table shows that Concentration 3 of Component C is required to protect *P. aeruginosa* from Antibiotic A within the base transport media. This is near the cutoff of where Component C concentration begins to affect filterability. Filterability was measured by following Protocol 1, in the Appendix, and having a transport time of 0. As seen in Table 2, below, filterability is severely impacted at higher Component C concentrations. Table 2, in conjunction with Table 1, show that an acceptable Component C concentration to afford protection from Antibiotic A and allow filtration is around Concentration 3.

Table 2. Filterability of fresh blood in transport media containing varying Component C levels

Component C Concentration Level	Filtration time
3 Component C	Failed
3 Component C	Failed
2 Component C	47 sec
1 Component C	32 sec
No Component C	33 sec

Another additive to the transport media is Component D. Component D has been shown to increase the degradation of Class B antibiotics, such as Antibiotic B. The effect of Component D on Antibiotic B is shown below, in Table 3. The effect of Component D on Antibiotic B can also be showcased chemically as seen in Figure 3. This data shows that Component D completely neutralizes Antibiotic B within the transport media.

Table 3. Cell viability with Component D and Antibiotic B.

Condition	Relative Cell Viability (%) After 4h Incubation
No Component D No Antibiotic B	395%
Peak Serum Antibiotic B	22%
0.8% Component D	469%
0.8% Component D Peak Serum Antibiotic B	277%

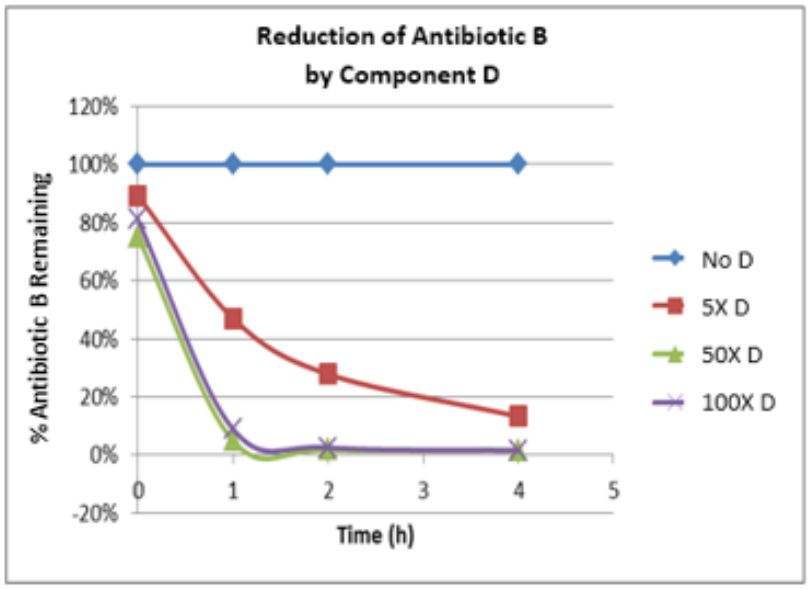

Figure 3. Chemical reduction of Antibiotic B by Component D, measured by absorbance (λ=520nm).

Following blood cell lysis, a protease is added to digest proteins within the blood serum and those released from lysed cells. This step is somewhat delicate, since the protease can also render the microorganism of interest nonviable. Since the protease activity is impacted by the temperature, incubation at a fixed temperature for a fixed time was selected to maximize both microorganism viability and sample filterability.

Once an initial transport media containing detergents for lysis, Component C for anticoagulation and Class A antibiotic protection, buffer, and minimal nutrients was shown to be capable of maintaining organism viability through protease digestion, testing was performed to quantify the impact of transport time. After a sample is collected from a patient, it could sit in the transport media for hours before it reaches the lab and protease digestion can be initiated. It is important to ensure that the organisms of interest remain viable and the sample remains filterable over this transport window. This media composition was tested to quantify the filterability of blood samples across a range of potential transport times. Testing was carried out by following Protocol 1, contained in the Appendix. Figure 4, below, shows filtration times for varying "transport time", Component C concentration, and blood donor. "Saline" indicates transport media containing nothing but detergents and minimal Component C. It is used as a control to show the filterability when no nutrients, buffers, or antibiotic inhibitors are present.

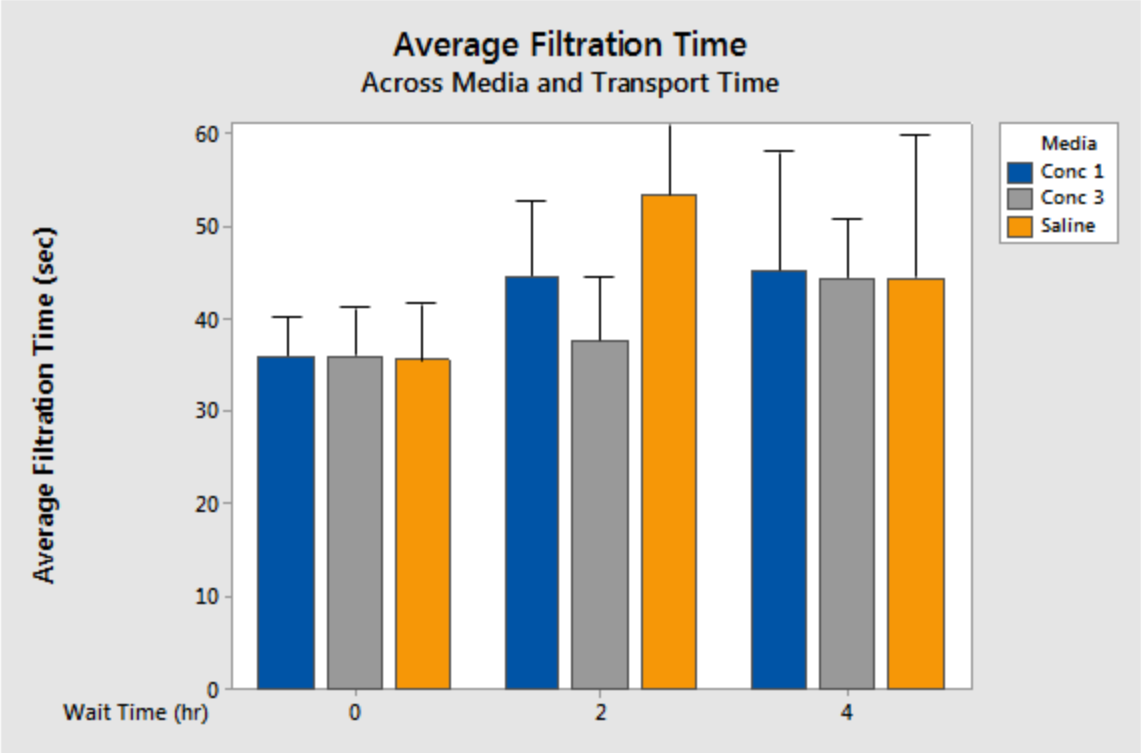

Figure 4. Filterability following transport of 0, 2, and 4 hours (n=9).

Samples must not only remain filterable, but must also maintain viable microorganisms. To determine viable organism recovery, different antibiotic-susceptible microorganism combinations were tested. To test this, transport media bottles were spiked with peak serum concentrations of certain antibiotics, and known quantities of susceptible bacteria, following Protocol 2, in the Appendix. The results, Figure 5, show that in the presence of certain antibiotics, viability is maintained and some organisms even begin to grow quite rapidly. However, certain drug-bacteria combinations are problematic, namely *E. faecalis*/Antibiotic C, *S. aureus*/Antibiotic D.

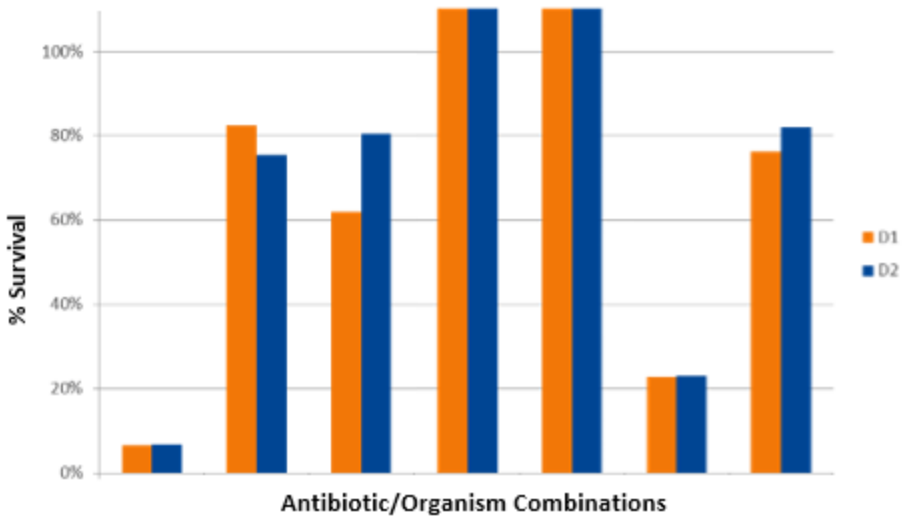

Figure 5. Microorganism growth within transport media in presence of antibiotics. Tested across two donors.

Filtration Geometry

To maintain simplicity within the device, dead-end filtration was chosen over other filtration techniques, such as tangential flow. However, there are still numerous geometries that can be implemented. The two geometries considered are shown in Figure 6.

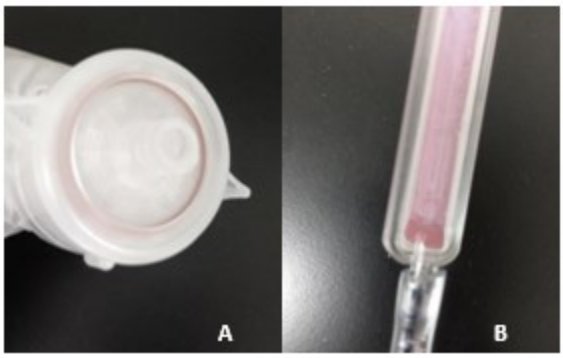

Figure 6. Different filter geometries. A) disk filter holder B) off-the-shelf vertical filter

Figure 6A shows a simple disc filter, where a pressure differential is created by applying a vacuum in the middle of one side of the filter and introducing a feed to the middle of the other side. Figure 6B shows a device containing vertical filtration geometry, where the

pressure differential is created by applying a vacuum at the top (back side) of the filter and introducing the sample at the bottom (front side) of the filter. This still involves the creation of a pressure differential across the membrane, but the vertical offset between the inlet and outlet creates a pressure drop down the backside of the filter as the membrane begins to foul. This can lead to residual material on the bottom backside of the filter that cannot be washed away.

Filter Membrane Material Selection

Once filtration geometry is selected, a membrane type must be chosen to enclose within the device. A number of different membrane materials are commonly used for biological filtration. These membrane materials include: polyethersfulfone (PES), polyvinylidene difluoride (PVDF), cellulose acetate (CA), and track-etched polycarbonate (PCTE). The chosen CA, PES, and PVDF membranes are all compromised of fibrous meshes, whereas the PCTE membrane consists of a relatively smooth surface that has cylindrical pores etched through it. Further, the supplied CA, PES, and PVDF membranes were all hydrophilic, compared to the PCTE membranes, which were available with a hydrophilic polyvinylpyrrolidone (PVP) coating, or in an uncoated state. Although the uncoated PCTE has a water contact angle slightly below 90^{o}, which is moderately hydrophobic, it is referred to as hydrophobic in order to differentiate the property difference between the coated and uncoated PCTE membranes. A pore size of 0.45 μm was chosen for the fibrous membranes, while a pore size of 0.40 μm was available for the track-etched membrane. Although 0.22 μm has become the standard for sterile filtration, the pathogenic

organisms of interest are all greater than 0.40 µm, and the larger pore size allows for easier filtration.

To quickly screen the above membranes, an experiment was designed to test the filterability of lysed and digested blood across each membrane using an effective filtration area of 13.2 cm^2. This area is much larger than the desired area within a consumable (~3–5 cm^2), so if a membrane struggles to filter at this surface area, it can be ruled out as a potential component of the final system.

For each sample, 10 mL of fresh blood was collected and processed according to Protocol 3, contained in the Appendix. A high vacuum pressure (-0.85 bar) was used, since any materials that fail at high pressure and high surface area will not suffice at the lower surface area, as desired in the final device. Table 4, below, shows the initial screening results of this experiment for 2 samples each of CA, PES, PVDF, hydrophilic and hydrophobic PCTE, and a control filter of the previous geometry. These initial results make it clear that the CA and PES filter membranes from this particular supplier do not work in this application.

Table 4. Filtration time across different membrane materials under high pressure and surface area

Membrane Materials Choice	Vacuum Pressure (bar)	Average Filtration Time (sec)
CA	0.85	DNF
PES	0.85	DNF
PVDF	0.85	16±9
PCTE (hydrophobic)	0.85	8±4
PCTE (hydrophilic)	0.85	15±8
Control	Peristaltic	47±9

The vacuum pressure was varied and the above experiment repeated for PVDF, hydrophilic PCTE, and hydrophobic PCTE. It is important to note that fresh blood samples were obtained from multiple donors and each membrane was tested with each donor. Since the properties of the blood from an individual donor impact filtration, it is important to control this during measurements. For a given donor, hydrophobic PCTE filtered over twice as quickly as the PVDF, while hydrophilic PCTE outperformed PVDF by an insignificant amount. The below plot, Figure 7, shows a comparison between the hydrophilic and hydrophobic PCTE. As shown in the plot, the hydrophobic membrane performs nearly twice as well at any given pressure. Also of note, the hydrophilic membrane experienced filtration failures at pressures as high as 0.60 bar, whereas the first failure for the hydrophobic membrane was at 0.40 bar.

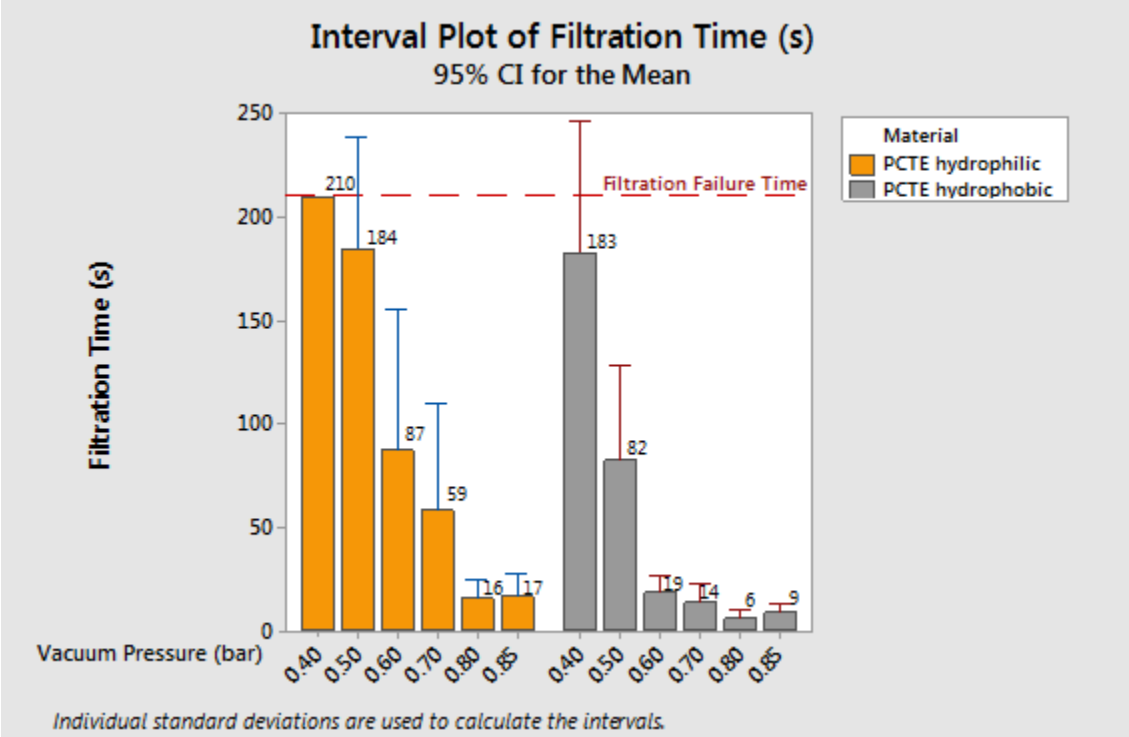

Figure 7. Filtration time for PCTE with hydrophobic and hydrophilic coating.
Note: Filtration time was capped at 210 seconds, meaning any sample that reached 210 seconds was considered a failure.

To further confirm the choice of hydrophobic PCTE as the filter membrane material, a 2-sample t-test was performed. This showed that at high pressures (> 0.60 bar) the difference between the mean filtration time using PCTE hydrophobic and PCTE hydrophilic membranes was statistically significant (p-value = 0.016). The variance between the two membrane materials was also compared. Figure 8 shows the results of this comparison and makes it clear that the hydrophobic membranes not only allow for faster filtration, but allow for more consistent filtration times. Consistent filtration times imply that the hydrophobic membrane is better suited to handle a broad spectrum of patient blood samples, since the hydrophobic membrane did not experience the effects of "difficult" donors as harshly as the hydrophilic membranes.

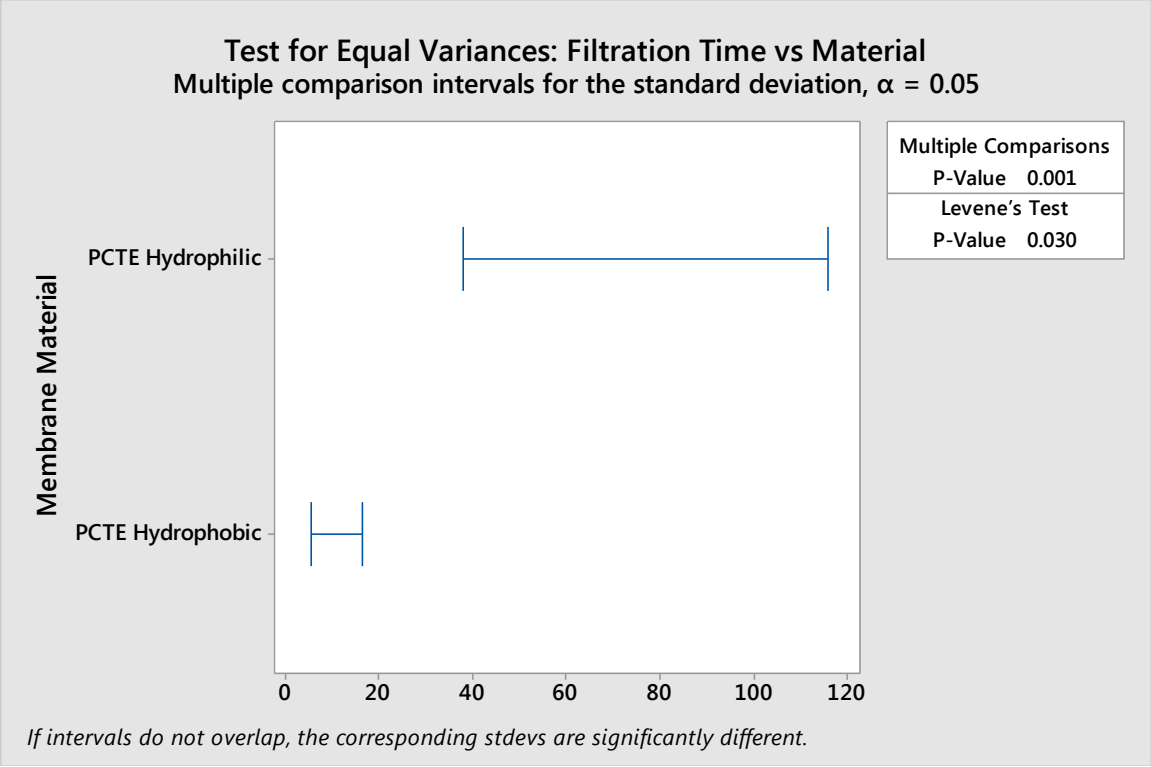

Figure 8. Test for equal variance in filtration time between PCTE hydrophilic and hydrophobic membranes.

Once the hydrophobic PCTE membrane was decided upon, more filtration tests were conducted using a much smaller surface area. Protocol 4 describes the process used to prepare and filter samples on a filter holder with an effective surface area of 3.5 cm^2. Figure 9 shows filterability results for these filters containing hydrophobic PCTE membranes. As seen in Figure 9, filterability is maintained with the lower surface area, and the average filtration time is roughly 1 minute.

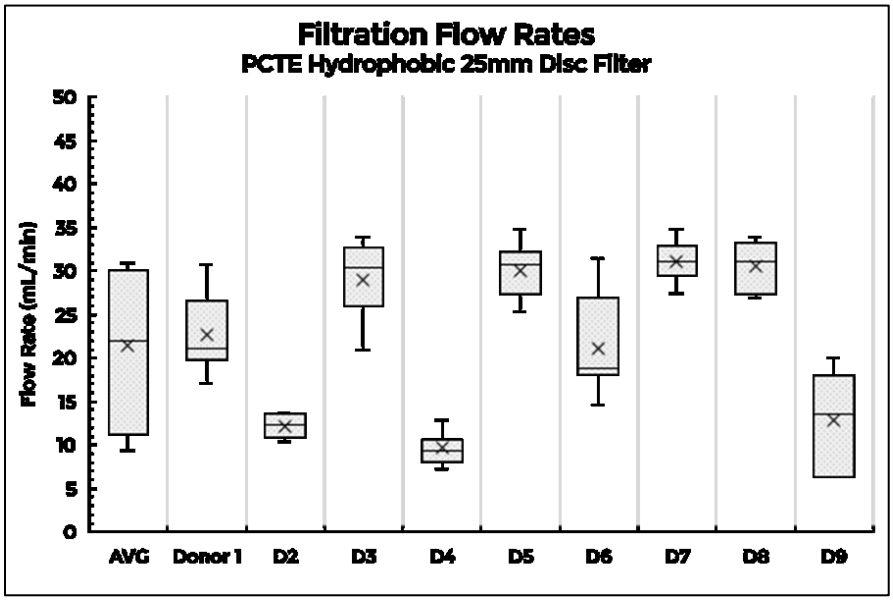

Figure 9. Filtration flow rates across 9 donors for 25-mm PCTE hydrophobic membranes. *Note: Samples were 22 mL, so filtration time = 22 mL / flow rate.*

To further characterize the fouling during filtration, SEM images were taken of the 25 mm filter membranes in different conditions. Figure 10, contains images of the membrane pores, both with and without debris from blood filtration. This figure also showcases the debris remaining on the surface of the filter following filtration via Protocol 4.

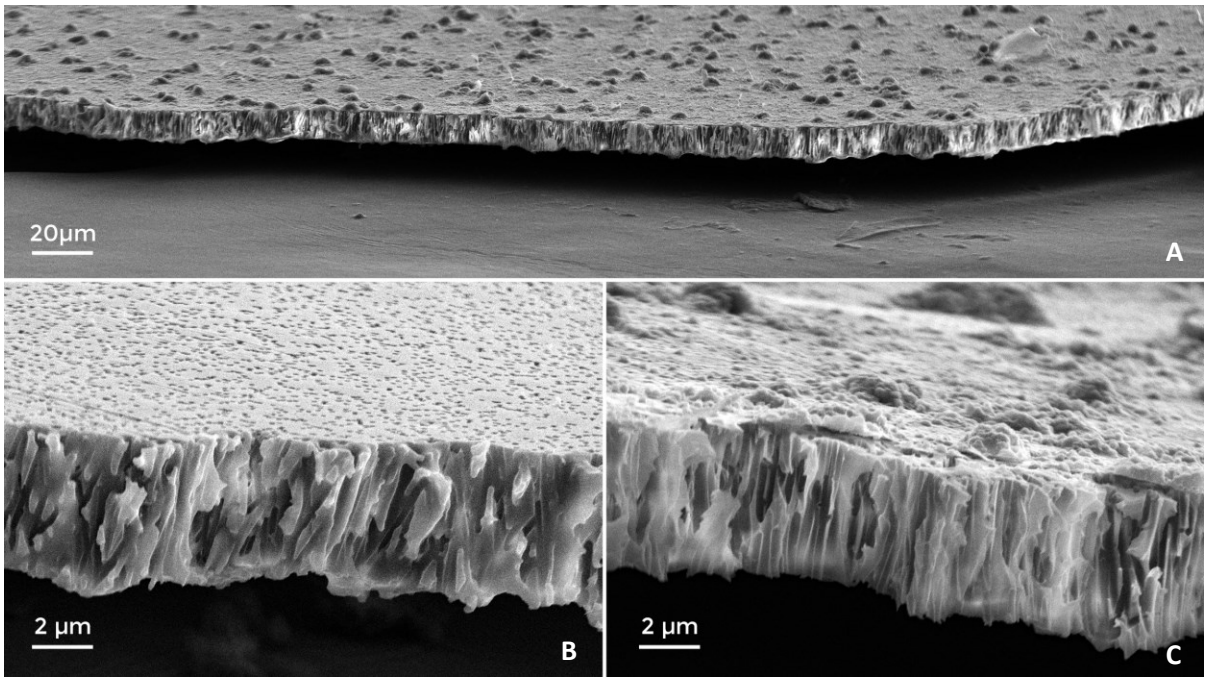

Figure 10. SEM images of hydrophobic PCTE filter A) surface containing debris B) freeze fractured pore cross sections (clean sample) C) freeze fractured pore cross sections (sample containing debris)

Detection Methods

After antibiotics and blood components are removed from a sample, there are numerous ways to detect whether or not growth occurs. The traditional method of detection used in automated blood culture systems, such as BACTEC, incorporate an indirect CO_2 sensor. This sensor indirectly measures the change in CO_2 within the sample vial and once the rate of CO_2 production increases to a certain point, the sample is signaled as positive. The rate of CO_2 production is indicative of growth because as aerobic organisms respirate, they convert O_2 into CO_2.

A simple experiment was performed to estimate the detection sensitivity of a BACTEC system. A known concentration of *E. coli* was spiked into a BACTEC bottle containing growth media and placed into the instrument and allowed to incubate until

detection occurred. Once the bottle was considered positive by the BACTEC, it was removed and the final *E. coli* concentration calculated by creating serial dilutions and inoculating BD TSA II 5% Sheep Blood plates with the sample. The growth curve generated by the instrument is shown below in Figure 11, which indicates the values of the starting concentration and most importantly the concentration at detection. The concentration at detection of 2.1×10^8 CFU/mL indicates that for bacteria that replicate and produce CO_2 at a similar rate to *E. coli*, detection will occur at a concentration on the order of 10^8 CFU/mL.

The detection time is based on algorithms, applied to the data by the BACTEC software, in order to indicate growth. In general, such algorithms are based on an acceleration of the signal, which is indicative of exponential growth. The concept of signal acceleration as a sign of exponential growth is applied in the following experiments in order to estimate positivity for setups, which do not contain tuned algorithms like the BACTEC. Simply, positivity is estimated based on when the curve of the growth signal appears exponential.

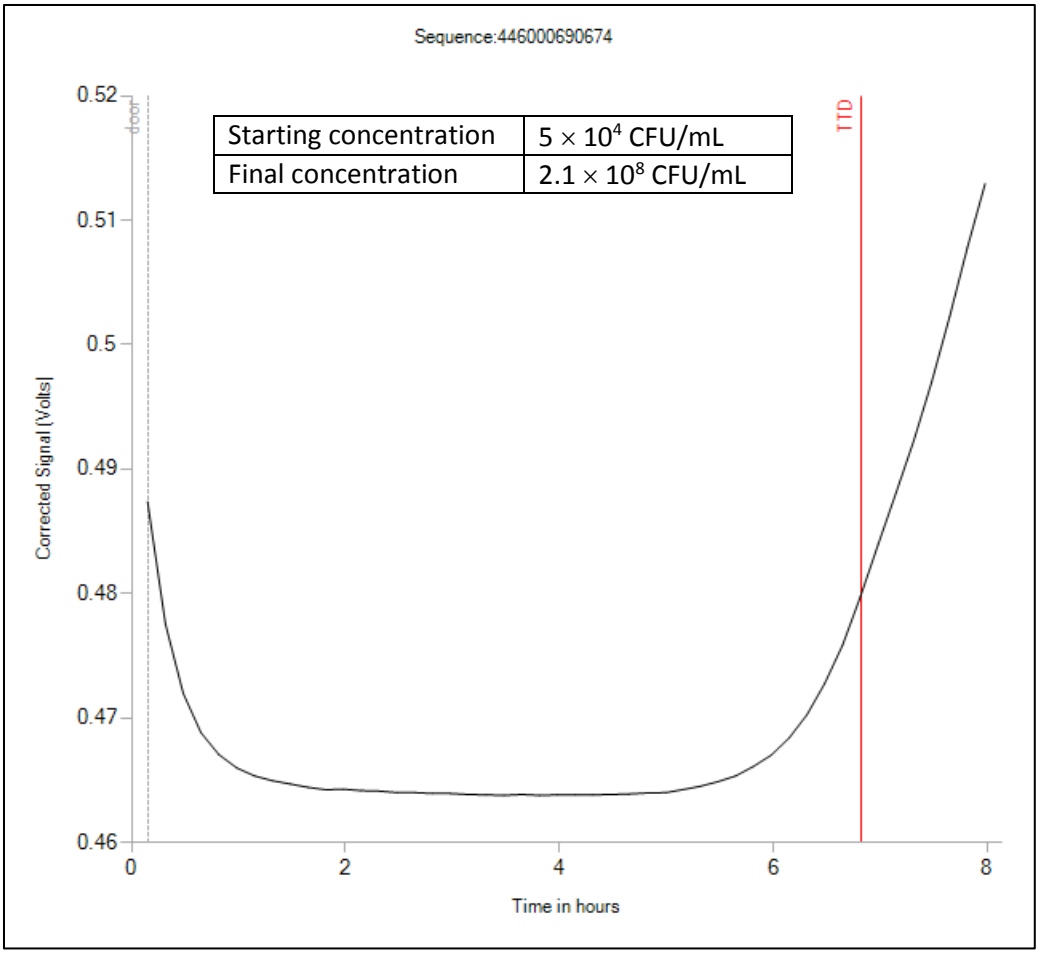

Figure 11. BACTEC generated growth curve of *E. coli*.

A potentially alternative method of detection is using a resazurin indicator. As the microorganisms proliferate in the presence of the indicator, resazurin is chemically reduced to resorufin. In its reduced state this indicator can be detected via fluorescence measurement.

An experiment similar to the one performed on the BACTEC was performed using the resazurin indicator and a fluorescence detector. A known concentration of *E. coli* was spiked into growth media containing indicator and placed into an incubator adapted to contain a fluorescence detector. The change in fluorescence was monitored and a detection time was estimated based on the curve. This data is represented below, in Figure 12. Since, this system does not have developed real-time detection algorithms, the samples were

removed roughly 15 minutes after the positivity point. Since *E. coli* has a generation time of a little more than 20 minutes, it can be estimated that the final concentration of 2.1×10^8 CFU/mL is no more than twice the actual concentration at positivity. This means that the sensitivity of resazurin-based detection is on the same order of magnitude as the BACTEC CO_2 mechanism (~10^8 CFU/mL).

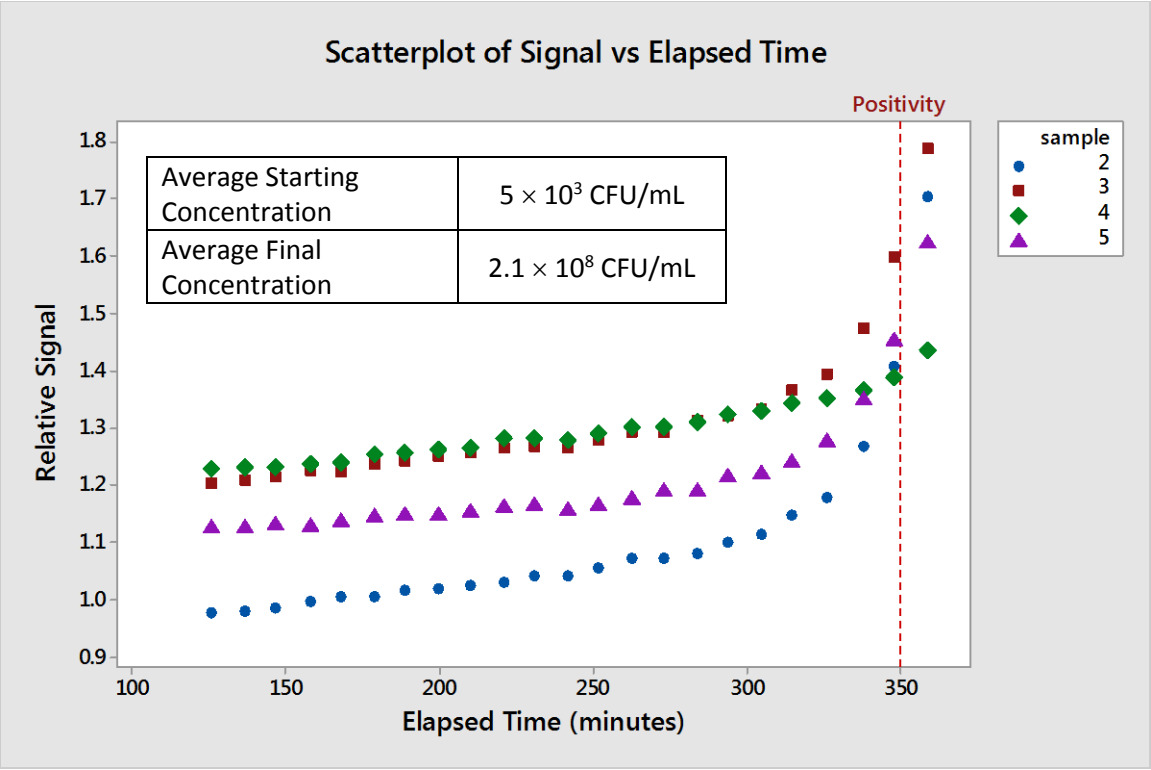

Figure 12. Growth curves of *E. coli* using resazurin detection.

The other detection method evaluated was nephelometry. A nephelometer employs a source beam and detector 90 degrees of the beam to grant a relative measure of the turbidity of a solution based on light scattering intensity. Meaning that as more particles are present in a suspension, more light is scattered leading to a higher output voltage. This does not rely on acceleration metrics like CO_2 production or resazurin reduction, so it is possible to simply add a known concentration of organism in growth media to a cuvette and place within a nephelometer to get an estimate of the sensitivity.

Table 5, below, shows sensitivities for numerous organisms, all estimated by adding known concentrations of organisms to a sample of growth media and comparing against a baseline reading of the same growth media. It is important to note that these estimates are in a pure sample of growth media, since filtration will undoubtedly leave some debris behind causing signal noise in the nephelometer readings. Further, these estimates are based on a signal increase of about 25 mV over baseline nephelometer readings. 25 mV is chosen because a signal-increase of >25 mV is indicative of growth and is not likely noise from the detector. The curve of *E. coli* growth monitored via the nephelometer, Figure 13, makes this 25mV cutoff more intuitive.

Table 5. Direct to nephelometer sensitivity estimates.

Organism	Sensitivity Estimate
E. coli	10^6 CFU/mL
C. albicans	10^5 CFU/mL
S. pneumoniae	5×10^6 CFU/mL
E. faecalis	5×10^6 CFU/mL
S. aureus	5×10^6 CFU/mL
P. aeruginosa	5×10^6 CFU/mL

The curve in Figure 13 was generated by spiking a known concentration of *E. coli* into a cuvette containing growth media, and then repeatedly recording the scattering. Like the developed resazurin system, this nephelometry setup does not have real-time detection algorithms; however, a sample was taken from the cuvette at 355 minutes, which is the estimated time to reach 10^6 CFU/mL. The resulting concentration of $1.5\text{x}10^6$ CFU/mL, and corresponding 20-25 mV signal increase over baseline, shows that experiments culturing *E. coli* within the cuvette agree with the separate spiking sensitivity estimates.

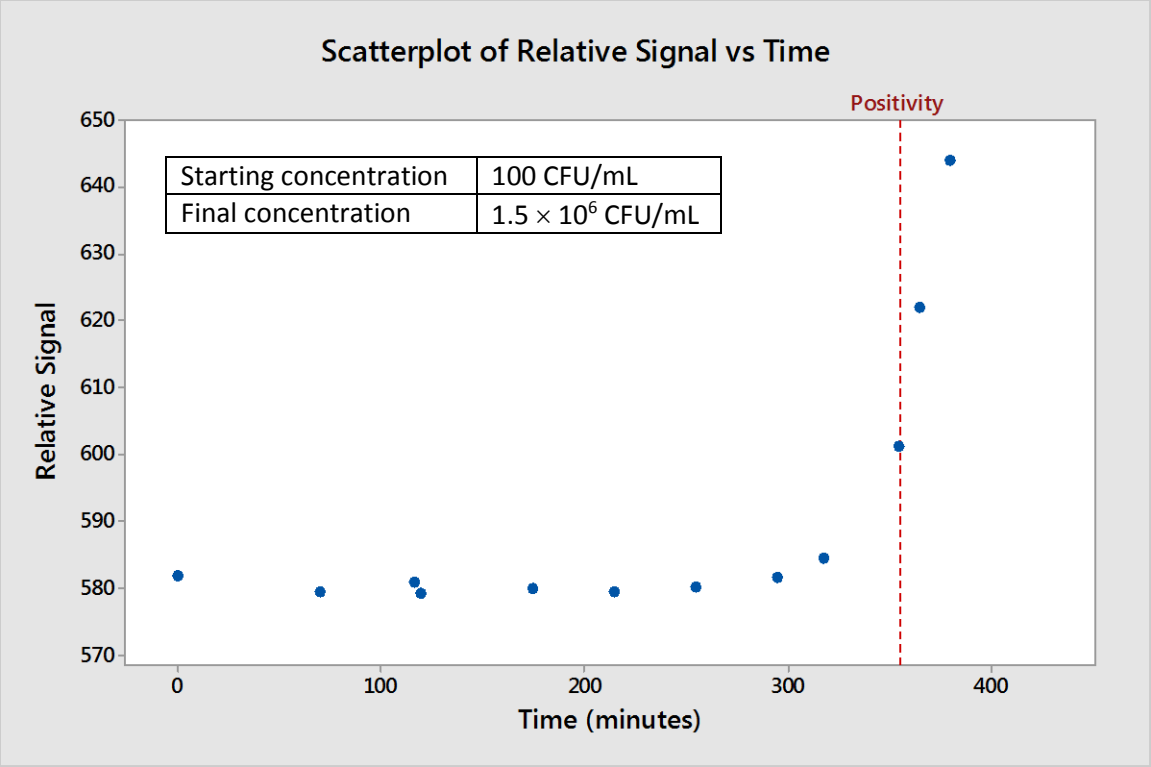

Figure 13. Growth curve of *E. coli*.

Curves were also generated for other organisms contained in Table 5. A sample of these other growth curves is shown in Figure 14. The concentration following a ~25mV signal increase for these organisms also fit well with the sensitivity estimates in Table 5. The growth curves in Figure 14 do not independently measure the sensitivity of the method, but rather verify the concentration required for a 25mV increase over baseline, previously estimated in Table 5. Further, these curves demonstrate additional noise that is not seen in the *E. coli* example. This added noise, likely due to the need to mix these samples before taking a measurement, shows a potential complication when filtration debris is introduced into the system.

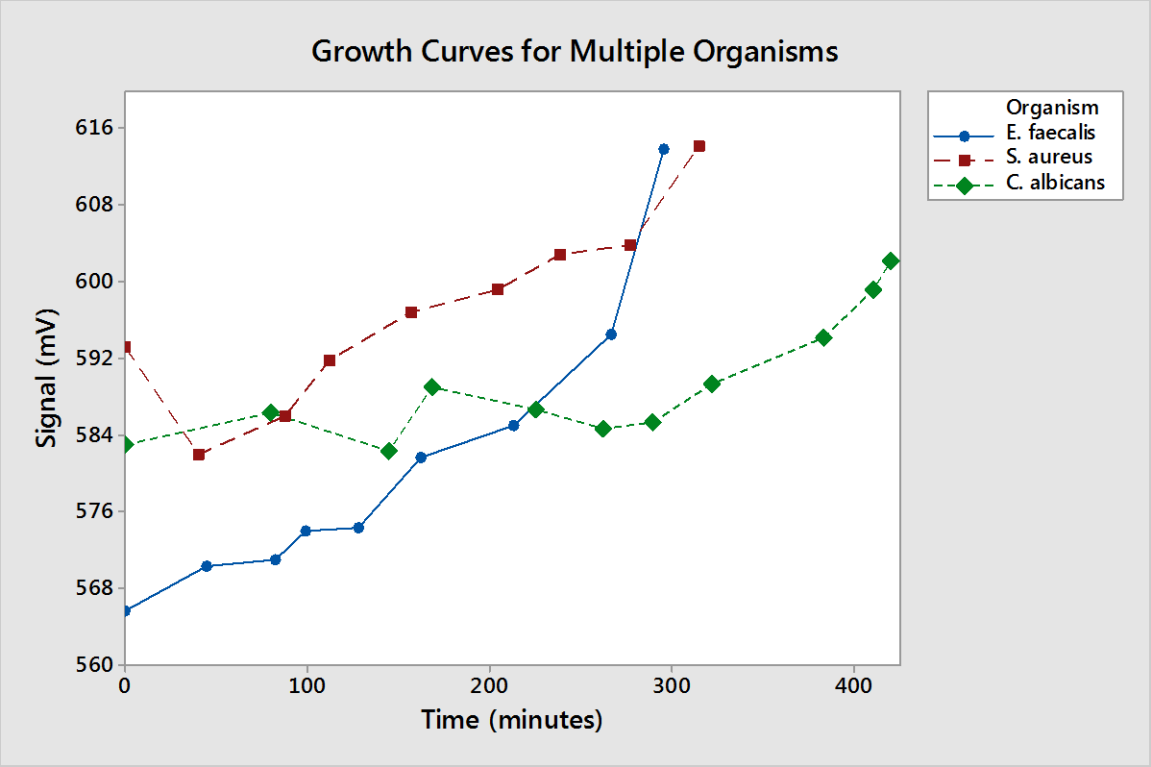

Figure 14. Growth curves for selected organisms.

In order to observe the effect blood debris will have on the noise of the nephelometer measurements, an experiment was setup to add debris to the cuvette to introduce the noise. The experiment, described in Protocol 5, contains all steps from blood collection to positivity. Since it has already been shown that the transport media will sustain relevant microorganisms through digestion when in the absence of antibiotics, the bacteria are spiked into the sample blood following digestion. Spiking in this manner does not allow the organisms to grow before filtration, so it is possible to approximate the number of organisms in the initial load within the cuvette. The data generated from this protocol is shown in Figure 15.

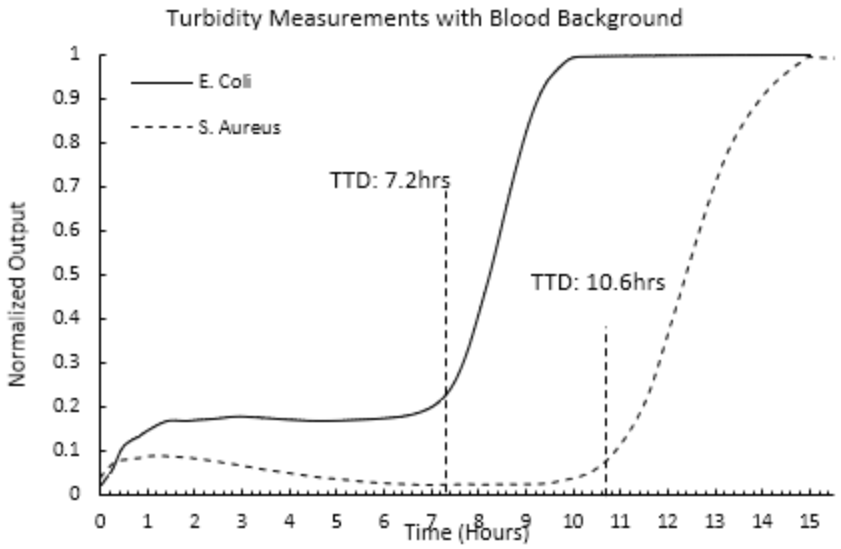

Figure 15. Nephelometer growth curves from entire process.

Fastidious Organism Growth

This filtration system rinses nearly all blood components from the filter surface ensuring detection via light scattering is possible without high interference. This is extremely important for detection, but the growth of a few organisms is dependent upon components of blood. The most clinically relevant of these organisms is *Haemophilus influenzae*. *Haemophilus influenzae* is reliant on two growth factors found in blood: Component A and Component B. Since these factors were thought to be removed via filtration, the growth media was supplemented with a concentration of Component A and a concentration of Component E. Component E is a precursor to Component B, which can be converted to Component B through salvage pathways in numerous microorganisms, including *Haemophilus influenzae*. Supplementation using Component E was chosen over Component B, since Component B is unstable in solution, which would lead to an unacceptably short shelf life of any growth media. Figure 16, below, shows growth results in media supplemented with differing concentrations of Component A and Component E.

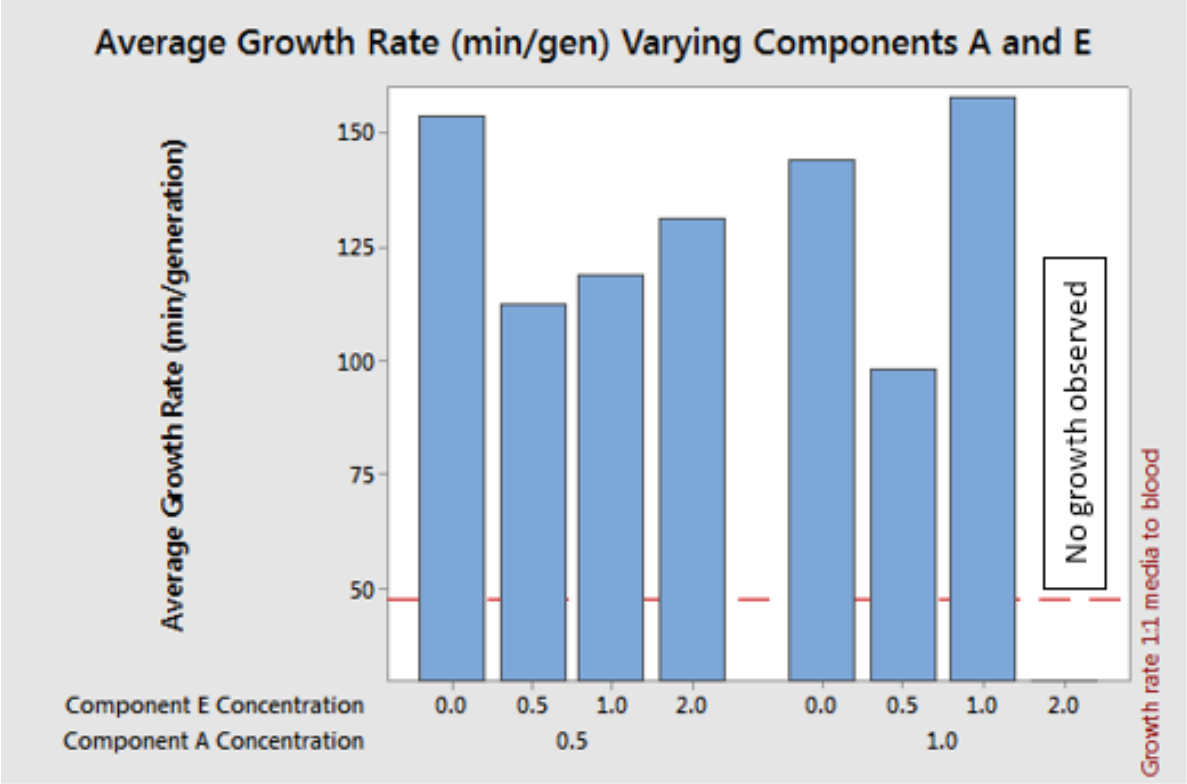

Figure 16. *H. influenzae* growth with varied concentrations of Component E and Component A. *The red line indicates the growth rate when H. influenzae is in a 1:1 mixture of blood and growth media. This is the target growth rate. n=2 for each condition.*

The above results indicate that the Component E was not being converted to Component B as quickly as necessary and resulted in growth rates of greater than 100 minutes per generation. Further, high concentrations of Component E, are known to inhibit the growth of *Candida albicans*, which is a clinically relevant infectious yeast.

Since Component E supplementation did not lead to acceptable growth rates, a few other alternatives were explored. Table 6, below, describes the formulations tested in Figures 17 and 18. Standard media is a basic growth media used in BACTEC systems, while the other media formulations were based on a higher nutrient media.

Table 6. Growth media formulation abbreviations used in Figures 16 and 17.

Abbreviation	Description
SA	Standard media
Supplement F	Media with Supplement F
Conc1 CompA	Nutrient rich media with added Component A
CompE	Added Component E
CompB	Added Component B

Figure 17, below, shows the growth rates of *Candida albicans* in different growth medias. This plot clearly shows the inhibitory effect of Component E on *C. albicans* growth. The other growth media all produced similar growth rates, with the nutrient rich formulations slightly outperforming the standard, regardless of extra additions such as Supplement F or Component B. This plot also shows that the media additives, other than Component E, did not affect the growth rate of *C. albicans*. Media with added Component B or Supplement F had rates essentially equivalent to the plain high nutrient media with Component A.

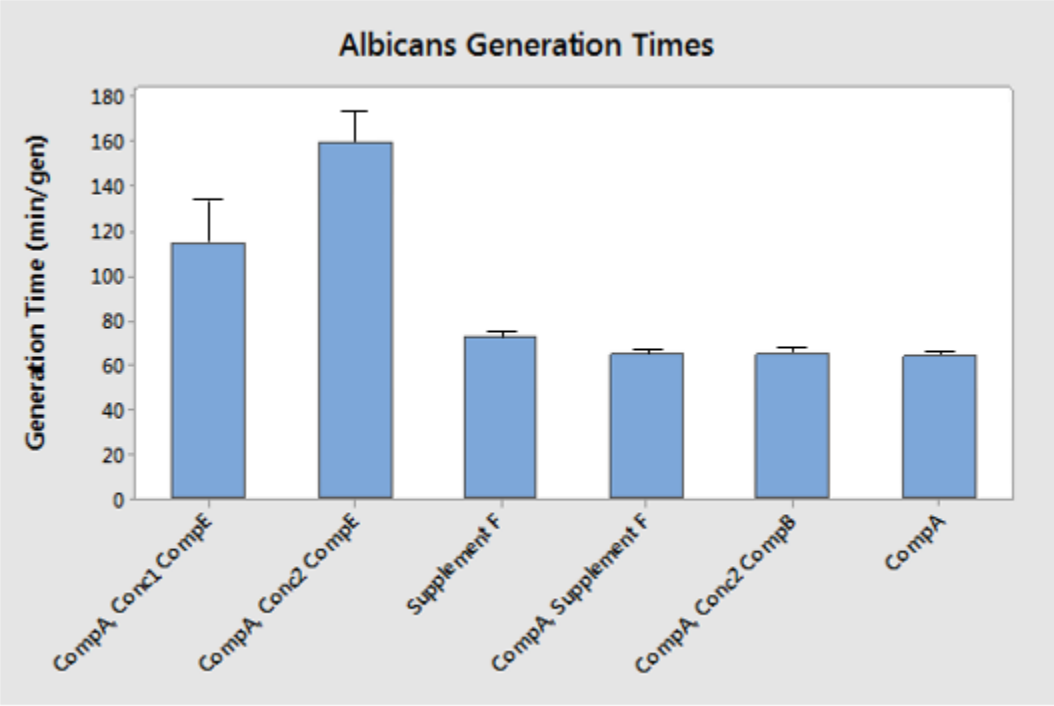

Figure 17. Growth of *Candida albicans* in different growth media (n=2).

Tests using a Component B spike, as opposed to Component E, led to a 3-fold faster growth rate (n=3), $p<0.05$. Spiking with small amounts of blood led to growth rates twice as fast as Component E spiking (n=3) $p<0.05$. Interestingly, filtration appeared to leave behind enough Component B to allow reasonable Haemophilus growth. Figures 18 and 19 summarize this data. Also of note, all of the growth work was carried out based on Protocol 6, contained in the appendix.

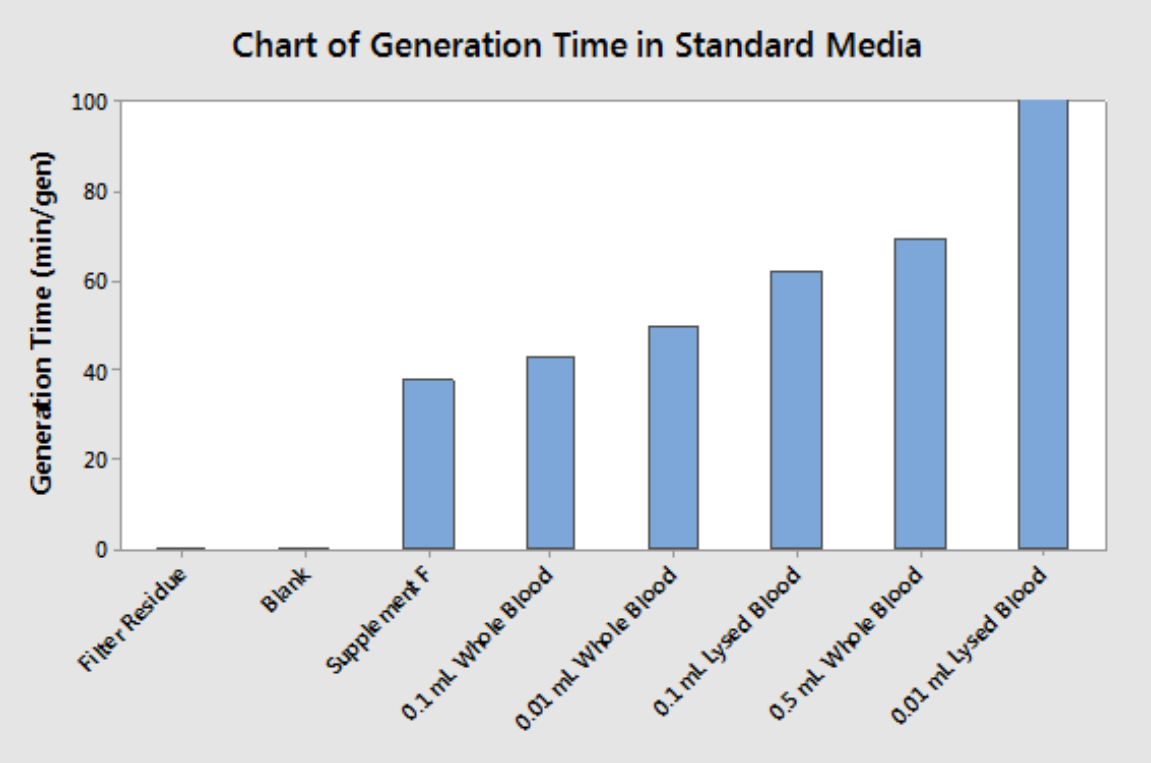

Figure 18. *H. influenzae* growth rates in standard media with different additives (n=3).

Within standard media containing supplement F, the bacteria grew three times faster than in media containing Component E (n=3), $p<0.05$. The difference in growth rate between bacteria growing in standard media supplemented with Supplement F and 0.1mL of whole blood was not statistically significant, $p>0.05$. The was no growth when only filter residue was added to the standard media. Generation times within standard media containing 0.01mL

of lysed blood were twice as long as those within standard media containing 0.01mL of whole blood (n=3), $p<0.05$.

H. influenzae appeared to grow much more quickly in the nutrient rich media with added Component A, shown in Figure 19. Most notably, nutrient rich media supplemented with Component A allowed growth to occur with only filter residue, whereas standard media did not support such growth. As expected the bacteria grew quickest when Supplement F and Component B were supplemented, with both producing a 3-fold higher growth rate than media supplemented with Component E (n=3), $p<0.05$. The difference in growth rate when both standard and nutrient rich media contain Supplement F was insignificant.

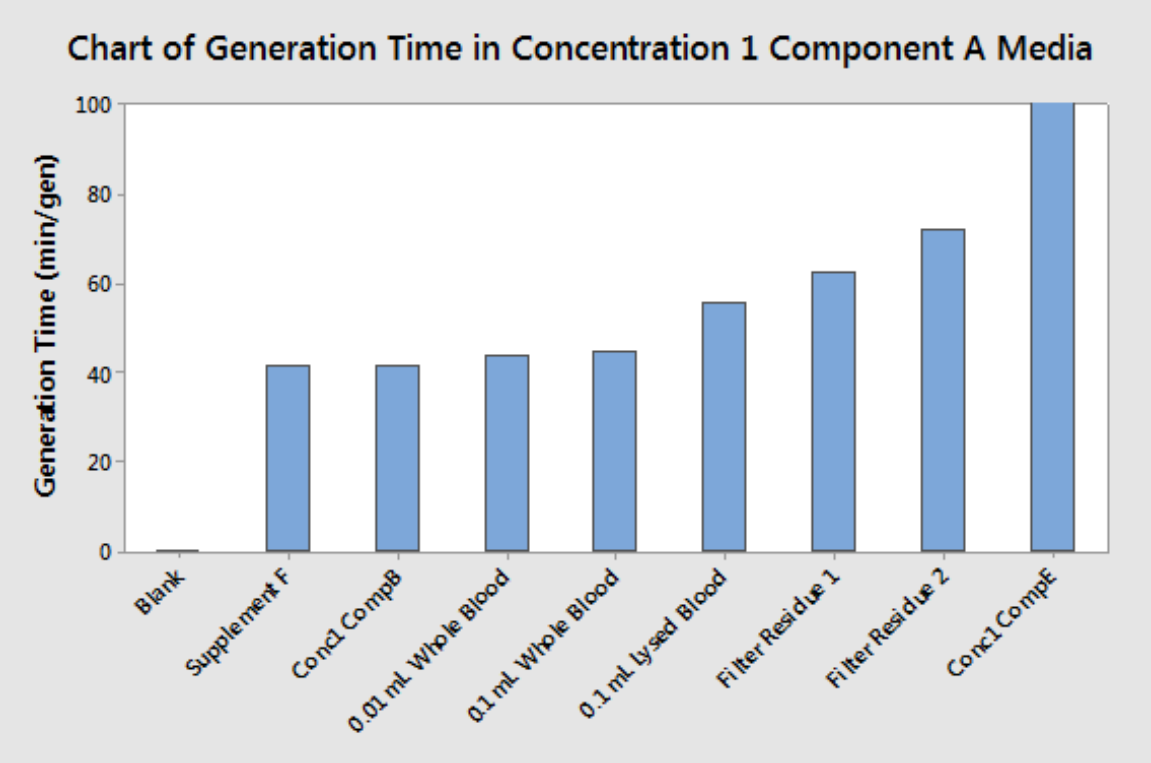

Figure 19. *H. influenzae* growth rates in nutrient-rich Component A media with different additives (n=3).

General Growth

Since each detection mechanism is reliant on microorganism growth, it is important to show that a representative array of organisms can be cultured within the developed nutrient rich media. Following Protocol 7, generation times of multiple organisms were calculated, as shown in Table 7. The effect of agitation on the growth rate is important because some detection systems are more amenable to agitation than others. It should be noted that these calculated generation times in the developed nutrient rich media, fit nicely with those found in the literature and historically in BACTEC media.

Table 7. Average generation time of organisms when incubated with or without agitation

Generation time in minutes/generation			
E. coli		***S. aureus***	
Rotating	**22.8±0.1**	Rotating	**30.0±1.1**
Stationary	**24.8±0.1**	Stationary	**33.5±1.6**
E. faecalis		***P. aeruginosa***	
Rotating	**22.5±0.3**	Rotating	**35.3±0.8**
Stationary	**24.1±0.4**	Stationary	**38.9±0.3**
C. albicans		***C. glabrata***	
Rotating	**71.9±0.3**	Rotating	**97.9±2.1**
Stationary	**90.6±4.5**	Stationary	**97.9±1.6**

Workflow

The final workflow for this system is similar to that stated in Protocol 5 and is showcased below in Figure 20.

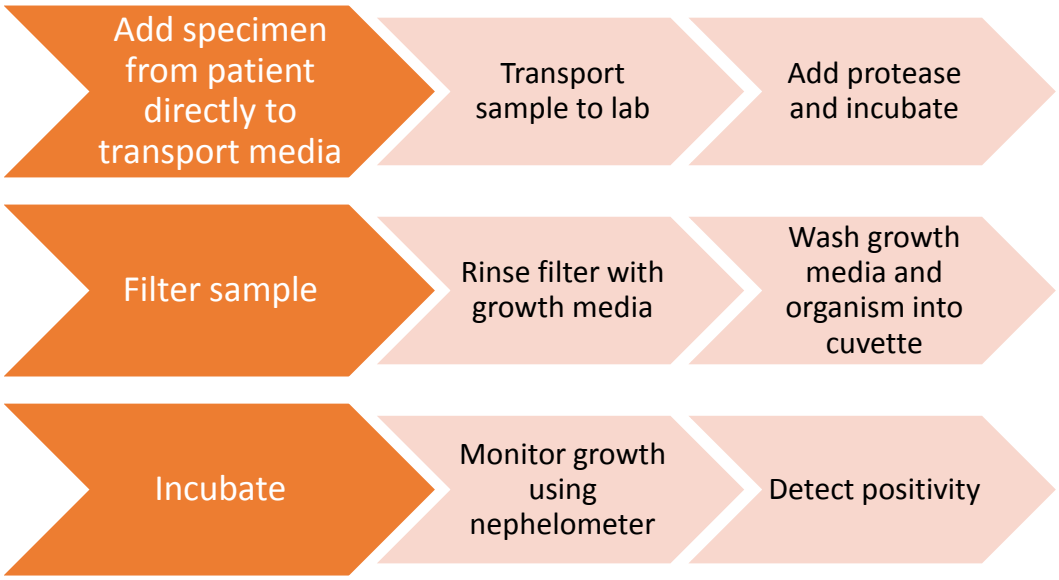

Figure 20. Workflow steps for developed system.

Discussion

Transport Buffer

The final transport buffer, composed of a number of proprietary ingredients, preserved organism viability for at least 4 hours in the absence of antibiotics. However, the presence of antibiotics greatly impacted organism viability in a few cases: e. faecalis/Antibiotic C and s. aureus/Antibiotic D, while others were protected against. Some of the protected cases include Component D reducing Antibiotic B and Component C protecting *P. aeruginosa* from Antibiotic A. Also able to survive a 4-hour transport window were the common combinations of: *E. faecalis*/Antibiotic E, *E. coli*/Antibiotic F, *S. aureus*/Antibiotic G, and *P. aeruginosa*/Antibiotic H. However, the inability to protect against all drug-microorganism combinations is a significant drawback that limits recovery when specific antibiotics are used.

Figure 4, in the transport media development section, showed that, with the addition of 0.5 mL of protease and a 1-hour incubation at 35ºC, sample filterability is maintained for at least 4 hours at the current composition. However, after 4 hours of transport, filterability becomes even more dependent on the patient, leading to filtration failure in some samples. This limits transport to a 4-hour window; however, blood culture transport times vary between hospitals, and while times can average as short as 40 minutes, other hospitals frequently take 24 hours to transport their blood culture samples to the lab.

There are a number of potential causes for the decrease in filterability over an extended transport time. One potential is that once the blood cells are lysed, the cellular components and membrane debris may begin to aggregate. Another potential issue is coagulation. Although Component C is an anticoagulant and is present in a concentration

greater than what is typically used to preserve blood, it is possible that micro-coagulates develop and are just large enough to block pores on the filter membrane. SEM images were taken in an attempt to better understand the causes of filtration failure. Figure 10, filtration development section, shows an SEM image of the filter membrane with and without lysed and digested blood cells. Although it is unclear what specifically is caked on to the membrane, it is likely that the cellular membrane was poorly solubilized. Poor solubilization of the membrane, in conjunction with the release of proteins during cell lysis, could account for the debris seen in the SEM image. Further, extended transport time could allow for large membrane/protein aggregates to form before the addition of protease. Then since the protease is only added for 1 hour, it is possible that it does not have enough time to degrade structures deeper inside of the aggregate.

Filtration Geometry

The simplistic nature of dead-end filtration led to its choice over tangential flow or other more complicated methods. Within dead-end filtration, different filter geometries were examined. Figure 6, showcases why the disc filter holder alignment was chosen over the rectangular preassembled vertical alignment. The vertical alignment allowed residual blood to remain on the back side of the filter, meaning that inhibitory factors or even antibiotics could remain in contact with the microorganisms of interest inhibiting their growth.

Further, the available filters in the vertical alignment are prefabricated and the housing layers are ultrasonically welded together, limiting the ability to change membrane materials. However, the horizontal disc filter holder is threaded, making it easy to interchange different membranes. The adjustability of the horizontal holder, coupled with the

ability to completely clear the backside separating all antibiotics and blood components, made it the clear choice of filtration geometry.

Filter Membrane

Figure 7 shows that uncoated PCTE is the clear choice of filter membrane. Uncoated track-etched polycarbonate (PCTE) allowed for significantly faster flow rates when filtering lysed and digested blood. Only two PCTE membranes were tested, one containing a hydrophilic PVP coating and one with no coating. Of the non-track-etched membranes tested, PVDF performed best, however, there was no further exploration of its properties since the uncoated PCTE allowed for filtration at much lower pressure differentials and had double the flow rate. It was surprising that PES performed so poorly, since PES membranes are low protein binding and are commonly used for filtering microbiology fluids, such as culture media. Similarly, the CA membranes, which failed at a large pressure differential (-0.85 bar), are marketed specifically for filtration methods requiring maximum protein recovery in the filtrate.

The high failure rate of low protein binding membranes indicates that released proteins in the sample were not likely the leading cause of filtration failure. They still may have aggregated to clog portions of the filter, but proteins should not have bound to these filter materials. This supports the theory that membrane debris is the leading cause of fouling and pore blockage.

Since different suppliers have their own proprietary manufacturing methods, it could be useful to see whether PES and CA filters from other suppliers also fail. It may also be beneficial to experiment with different coatings to change the surface properties of the filter

material to allow for greater flow rates under lower pressures, or to alleviate the need for such a long digestion process. Although it appears that improving membrane solubilization is the clearest path forward to improve filterability.

Detection Method

Nephelometry is shown to have a much better sensitivity than resazurin and CO_2 sensing. Depending on the microorganism present, the signal change from nephelometry can be distinguished from noise at anywhere between 10^5 CFU/mL and 10^7 CFU/mL. This compares quite favorably to resazurin and CO_2 sensing which have sensitivities 10–100-fold higher than nephelometry. Using a nephelometer to detect growth within the culture has a few drawbacks. Notably, the filtration process must remove nearly all of the digested blood components to avoid creating too much background noise. Further, the current setup requires agitation for detection purposes, since some microorganisms settle and do not scatter the incoming light. This adds an additional engineering challenge to create a complete system; however, as Protocol 5 and Figure 15 show, it is possible to implement an agitation mechanism by using a pump to pipette the microbial solution in order to agitate and promote gas exchange.

Figure 15 is also important because it follows the entire system workflow and demonstrates detection times achieved using a starting organism load between 10-100 CFU. This is important because it verifies a calculated comparison between traditional BACTEC time to detection and the time to detection of the new system. Table 8, below, shows the calculated comparison. Figure 15 showed that starting with ~30 total CFU of E. coli and S. aureus, gave TTDs of 7.2 hours and 10.6 hours respectively. The projection for *E. coli* (7.0

hours) fits very well with the TTD seen when testing the system. Although the projection for *S. aureus* (9.4 hours) is not quite as close, it is still within roughly 10% of the experimental TTD. Also, it is important to note that the testing setup involved eluting the bacteria from the filter in order to culture within a cuvette. This undoubtedly reduced the number of bacteria in culture, which could help explain why the theoretical times are slightly faster.

Table 8. Projected TTD for array of organisms.

Organism	BACTEC historical TTD (hours)	Projected TTD (hours)
Escherichia coli	11.0	7.0
Enterococcus faecalis	11.2	7.1
Staphylococcus aureus	14.0	9.4
Pseudomonas aeruginosa	16.4	11.1
Candida albicans	23.5	16.4
Haemophilus influenzae	23.0	22.0
Candida glabrata	47.2	24.0

Table 8 was constructed by simply stacking the TTD savings afforded by this system. The savings from volume reduction is strictly mathematical. A BACTEC bottle contains roughly 40 mL total volume, meaning that an initial load of 30 CFU begins at a concentration of 0.75 CFU/mL. In the devised system the volume in the cuvette is 2 mL (and can easily be reduced further), so an initial load of 30 CFU begins at a concentration of 15 CFU/mL (assuming all organisms are recovered in the cuvette). The other time savings is due to the increase in sensitivity of the nephelometer over the indirect CO_2 sensing. For the BACTEC, the bacteria must proliferate to increase their concentration from 0.75 CFU/mL to the detection threshold which is on the order of 10^8 CFU/mL for bacteria such as *E. coli*. Detection via nephelometry is more sensitive, so that bacteria must reach a threshold around $5x10^6$ CFU/mL and yeasts must reach roughly 10^5 CFU/mL. In this system the organisms of

interest begin at a higher concentration and must reach a lower concentration than in previous systems.

The calculations to develop the projected TTDs of this system are based on two equations. The first is used to calculate the number of doublings needed to reach the detection threshold of the nephelometer: $[final] = [initial] \times 2^{number\ of\ generations}$. Once the number of generations required is calculated, simply multiply by the generation time to calculate the time to detection: $TTD = number\ of\ generations \times generation\ time$. In projecting the system TTDs, sensitivity estimates, such as those in Table 5, were used, but had roughly 40% added to them to help account for the higher threshold when noise is present from the filter debris. The generation time in the presence of filter residue was used for H. influenzae, and times from Table 7 were used for the rest of the organisms.

The projections in Table 7 are extremely impressive. The largest reduction in TTD occurs for organisms with slower growth rates, such as the Candidas. *C. glabrata* has a projected time savings of nearly an entire day, although this organism is known to settle out of suspension, so it would be interesting to test this organism according to Protocol 5 to see if the projections still hold. Also of note, despite the *H. influenzae* growth rate being suboptimal, it still is projected to perform on par with the historical BACTEC standard thanks to the volume reduction.

Fastidious Growth

Since the filtration system removes nearly all blood components from the filter surface, it is necessary to ensure organisms that require certain blood components are still able to grow. *Haemophilus influenzae* is the most common of these organisms and is

dependent on Component A and Component B, both found in blood. Figure 16 shows that using the Component B precursor, Component E, is not effective for the culture of *Haemophilus*. This was unexpected since many commercial blood culture systems are supplemented with Component E. However, these systems all maintain the presence of whole blood throughout the culture process, so it is likely that the added Component E is having little effect on *Haemophilus* growth in such systems.

Supplementation with Supplement F performed extremely well, as expected, but the *H. influenzae* growth in the presence of just the filter residue was extremely surprising. This is even more surprising given that no growth at all occurred in the nutrient rich Component A media when it was not supplemented, shown as column "blank" in Figure 19. This implies that enough Component B, or precursors allowing for the synthesis of Component B, remains on the filter to support growth of *H. influenzae* at a reduced rate. The residual Component B is likely due to protein-bound Component B not being able to pass through the filter pores, which supports protein aggregation as a cause of filter clogging. *H. influenza* is rarely the causative agent in sepsis, so this reduced rate may be acceptable given overall improvements given by filtration. However, it is worrisome to rely on the filter to retain needed nutrients, and would likely be better to add fresh Component B to a growth media stock as needed.

General Growth

The growth rates of all non-fastidious organisms in the absence of antibiotics should be equal to or better than the rates in a traditional blood culture system. This is because the basis of the growth media is a high performing automated culture media; however, for this system certain nutrients could be increased without worry of interfering with the detection

mechanism. Further, the removal of all inhibitory blood components, while supplying those that are necessary, will likely increase the growth rate of microorganisms that are inhibited by such components.

Workflow

The workflow for this device is much more involved than the traditional blood culture workflow. In its current form the workflow presents too many opportunities for contamination or user error. However, the principles applied in this workflow can be implemented in a closed device. Future iterations should combine and automate steps to ensure a closed system with minimal likelihood of contamination or room for user error. One possible embodiment is an inclusive filtration/detection concept. In this concept, the steps following the lysis and digestion of the sample are all contained within one simple device. Media can be drawn in behind the digested blood and then filtered and transferred to a cuvette, without the possibility of contamination or loss of organism.

This concept describes a simple closed system from the point of blood digestion onward, but transport is also an issue. A potential solution for the issues presented by transport is to have a reusable sample transport system which integrates a collection device with the filtration and detection concept. Such a system could be comprised of a portable incubator containing filtration consumables. When a sample is added, a timer on the incubator would start, and following an hour digestion period, the filtration system would be activated, allowing automated control of the filtration steps.

Having a reusable device that would allow immediate addition of protease and start of incubation would result in a massive time savings. Instead of up to 24 hours of wasted time

during transport, a sample could be digested and filtered within the first hour of transport, leaving the remaining transport time as additional growth time. This savings of previously lost time, would allow many samples to arrive at the lab immediately prepared for identification and antibiotic susceptibility testing. This would amount to a TTD reduction of the transport time for every sample, in addition to the savings already afforded by decreased volume and a more sensitive detection mechanism.

Conclusion

The designed device allows for the collection, transport, filtration, incubation/growth, and detection of infectious microorganisms contained in blood samples of potentially septic patients. Within a 4-hour transport window and in the absence of Antibiotic D and Antibiotic C, this device significantly outperforms the current blood culture standard, reducing time to detection by hours across organisms. However, these are relatively stringent constraints. The transport time constraint cannot be met by all hospitals, which would undoubtedly lead to filtration failures causing samples to be inconclusive, further delaying identification and antimicrobial susceptibility results. Also, the transport media struggles to protect against Antibiotic D or Antibiotic C, so patients receiving these antibiotics would be at an increased risk of receiving a false negative.

There are a number of ways to address these deficiencies of the current device. To escape the issues of extended transport, samples could be placed into a transport container, which performs multiple functions to prepare and preserve the sample. Such a transport container would also alleviate some issues with antibiotics as well. By filtering within 1 hour the media would only have to protect the microorganisms for a fraction of the time and certain antibiotic-microbial combinations that were unrecoverable at 4 hours would once again become recoverable. Such a method would significantly decrease time to detection, while also improving recovery.

Another solution, which removes the drawbacks of transport, would be to move towards a near-patient model where the blood sample is filtered without a dependence on time and temperature. The workflow of such a system would be more straightforward than a reusable carrier and would allow immediate separation of the microorganism from any

inhibitory factors and antibiotics; however, this would require a rework of the transport media and digestion procedure. Near-patient filtration would be a difficult task, however, the foundation of the current filtration method and in place downstream processes, could make this a viable path.

Appendix

Protocol 1: General Fresh Blood Filtration (Vertical Filter Method)

1. Draw 10 mL of blood from donor into a 10-mL syringe.
2. Inoculate fresh blood into bottle of transport media.
3. Allow bottle to sit on benchtop for desired "transport time".
4. Following transport, add 200 units of protease A.
5. Place sample bottle in a rocking incubator at 35ºC for 1 hour.
6. After incubation, remove bottle from incubator, de-cap
7. Attach vertical filter to peristaltic pump and lower filter tip into the sample.
8. Turn on the pump and record the time for the sample to completely filter.

Protocol 2: Antibiotic-Susceptible Microorganism Transport Media Testing

1. Prepare a solution of the antibiotic of interest.
2. Prepare a 1.0 McFarland suspension of organism and dilute to desired concentration.
3. Take a sample from this suspension and plate to ensure there is no contamination and the sample concentration is accurate.
4. Add antibiotic to transport media, in a concentration equal to expected peak serum level.
5. Add desired quantity of the microorganism dilution to the sample.
6. Sit on benchtop for 4 hours and then add 200 units of protease A and incubate at 35ºC for 1 hour.

7. Remove from incubator and sample from each bottle, making necessary dilutions before inoculating several plates.
8. Once organism has grown on the initial and final plates, calculate the recovery.

Protocol 3: Filter Membrane Characterization

1. Add 10 mL fresh blood sample to transport media.
2. Immediately add 200 units of protease A and incubate at 35^{o}C for 1 hour.
3. Prepare filtration setup by attaching 47-mm disk filter membrane to manifold filter holder.
4. Close manifold.
5. Attach vacuum pump to manifold and adjust vacuum pressure to desired amount.
6. After incubation, pour sample onto open filter holder.
7. Open the manifold and start timer.
8. Once sample has been cleared from filter surface, stop timer.
9. Repeat using a new filter membrane and new disposable filter holder.

Protocol 4: 25 mm Filter Holder Characterization

1. Add 10mL fresh blood sample to transport media.
2. Immediately add 200 units of protease A and incubate at 35^{o}C for 1 hour.
3. Prepare filter holder by placing hydrophobic PCTE filter membrane inside filter holder.
4. Attach filter to vacuum pump and set vacuum pressure to -0.90 bar.

5. After incubation, use a 30-mL syringe to remove entire sample from transport media bottle.
6. Attach syringe to filter holder.
7. Turn on vacuum and begin timer.
8. Stop timer once entire sample has filtered.

Protocol 5: Nephelometry Detection, Sample Collection through Positivity

1. Collect 10 mL of fresh blood from donor and add directly to transport media.
2. Allow transport media to sit on benchtop for 1 hour.
3. Add 200 units of protease A and incubate at 35^{o}C for 1 hour.
4. Prepare 25 mm filter holder by inserting hydrophobic PCTE membrane and connecting to vacuum pump.
5. After incubation, spike ~30 CFU into lysed and digested blood sample.
    a. ~30 CFU obtained by creating 1.0 McFarland and diluting 10^6 times and then adding 0.1 mL of the 10^6 dilution.
6. Remove entire sample (~22 mL) from bottle using a 30-mL syringe.
7. Connect syringe to filter and turn on vacuum pump to -0.90 bar.
8. Once entire sample has filtered, disconnect 30 mL syringe and connect 10 mL syringe filled with growth media.
9. Allow entire syringe of growth media to rinse through filter.
10. Disconnect filter from vacuum pump and connect 5 mL syringe containing growth media to the filtrate side of the filter.
    a. Attach opposite side bacteria captured on.

11. Backflow 2 mL from the filter and collect in polystyrene cuvette.
12. Place cuvette inside a nephelometer within an incubator.
13. Connect a syringe pump, programmed to aspirate and dispense 0.5 mL of sample.
    a. This mixing keeps the particles from settling.
14. Allow to incubate at 35^{o}C until change in curve indicates positivity.

Protocol 6: Growth Media Studies

1. Add 5 mL of base media to 15 mL conical tube.
2. Add any additional supplements to desired concentration.
    a. i.e. Component E, Component B, Supplement F, whole blood, lysed blood, filter residue.
        i. Lysed blood acquired by following Protocol 1 through step 5.
        ii. Filter residue acquired by following Protocol 5 through step 11, but collecting eluate in with growth media instead of in cuvette.
3. Spike known concentration of *H. influenzae* or *C. albicans*.
4. Place on rotisserie rotator inside of incubator set to 35^{o}C.
5. After ~16 hours remove from incubator and dilute and plate.
    a. Make 10x serial dilutions by adding 0.5 mL of preceding dilution to 4.5 mL of sterile saline.
    b. Plate 0.1 mL of each dilution on appropriate agar plate.
6. Count plates and calculate final concentration and overall generation time.

Protocol 7: Organism Growth Testing With or Without Agitation

1. Prepare McFarland standard for each organism to be tested.
2. Dilute the standards for bacteria 10^6 times and those for yeasts 10^4 times. Plate final dilutions to ensure concentrations are known and there is no contamination.
3. Add 16 mL of nutrient rich media to 50 mL conical tube for each organism to be tested.
4. Add 2 mL of the diluted organism to a tube; repeat for each organism in a different tube.
5. Vortex to mix.
6. Take six 1-mL samples from each 50-mL tube and dispense into 1.5-mL tubes. Generate six 1.5-mL samples for each organism.
7. Place three samples from each organism into a rotating mixer (~25 rpm).
8. Place other three samples from each organism on stationary tray.
9. Place mixer and tray inside incubator at 35ºC.
10. Based on known generation times of the organism calculate length of time to ~10^6 CFU/mL for bacteria and 10^5 CFU/mL for yeasts.
11. Use these calculated times to remove samples at the proper time.
12. Dilute samples and inoculate plates.
13. Count plates and calculate final concentration
14. From the final concentration and initial concentration calculate the estimated generation time.
    a. $[final] = [initial] \times 2^{number\ of\ generations}$
    b. $generation\ time = \frac{total\ time\ incubating}{number\ of\ generations}$

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Curriculum Vitae

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EDUCATION

Johns Hopkins University, Baltimore, MD **December 2017**
Master of Science in Engineering, Materials Science and Engineering

Johns Hopkins University, Baltimore, MD **May 2016**
Bachelor of Science in Materials Science and Engineering
concentration: Biomaterials
GPA: 3.51 (Dean's List: Fall 2014 – Spring 2016)

EXPERIENCE

Becton Dickinson Sparks, MD **2016-Present**
Systems Engineer
- Designed and followed robust test methods to characterize complex instrument function
- Prepared detailed technical reports utilizing statistical methods
- Created testing protocols for verification and validation
- Collaborated cross-functionally to ensure technical solutions meet product specifications and requirements

R&D Engineer
- Collaborated cross-functionally in development of novel sepsis diagnostic device
- Coordinated efforts to establish filtration membrane material and geometry for consumable, doubling previously achieved flow rates
- Designed and led studies to develop and characterize new detection system, leading to 100-fold increase in sensitivity over previous standards

Johns Hopkins University Hai-Quan Mao Lab **2015-2016**
Research Assistant
- Fabricated and tested novel Tuberculosis testing device
- Performed analysis of testing device efficiency
- Analyzed reactions between thermites and Iodic Acid to optimize reaction for most efficient production of Iodine gas
- Utilized optimized thermite-iodic acid reactions for spore killing applications
- Performed literature reviews to establish protocols
- Developed and followed protocol for electrospinning HA nanoparticles within PCL fibers
- Created coating to enhance integration of bone into implant devices

Bayer HealthCare Myerstown, PA **2013-2014**
Quality Scientist
- Tested materials to USP specifications
- Performed UV-VIS, IR, NIR, HPLC testing
- Prepared samples for offsite testing, logged samples, and maintained testing records
- Prepared monograph revisions
- Increased efficiency with numerous database optimization projects

TECHNICAL SKILLS
- Minitab, Cognition Cockpit, SOLIDWORKS, MATLAB**,** JavaScript**,** LabVIEW, Mathematica**,** SAP, SQL, Microsoft Office (Word, PowerPoint, Excel, Outlook), Microsoft Project
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